

for the required fee of \$460.00, is submitted herewith, making the response due on January 29, 2003. Applicants respectfully request reconsideration and withdrawal of the rejections in view of the following amendments and remarks.

A version of the amended paragraphs of the specification is attached hereto as Appendix A, and a version of the amended claims is attached hereto as Appendix B, with the changes made by the present amendment indicated in bold.

## AMENDMENTS

### **Amendments to the Specification**

**Please replace the paragraph at page 1, lines 5-7 of the specification with the following paragraph:**

This application is a §371 national phase application from International Application No. PCT/US98/13007, filed June 22, 1998, which is a continuation-in-part application of U.S. Application Serial No. 08/879,565, filed June 20, 1997, now U.S. Patent No. 6,093,573, which is hereby incorporated by reference in its entirety.

**Please replace the paragraphs at page 14, lines 7-30, and page 15, lines 1-28 with the following paragraphs:**

**Fig. 1A** A ribbon diagram of residues 1-456 of BPI illustrating its boomerang shape. The NH<sub>2</sub>-terminal domain is shown; the COOH-terminal domain and the two phosphatidylcholine molecules are shown. The linker is also shown, and the disulfide bond is shown as a ball-and-stick model. **Fig. 1B** View after rotating Fig. 1A 70° about the long axis of the molecule. Figure produced with MOLSCRIPT [P. Krauliz, *J. Appl. Cryst.*, 24:926 (1991)] and RASTER3D [E. A. Merrit and M. E. P. Murphy, *Acta Crystallogr.*, D50:889 (1994); D. J. Bacon and W. F. Anderson, *J. Mo. Graphics*, 6:219 (1988)].

**Fig. 2A** Schematic drawing of the novel BPI domain fold, shown in same orientation as the NH<sub>2</sub>-terminal domain in Fig. 1B. **Fig. 2B** Superposition of the NH<sub>2</sub>- and COOH-terminal domains of BPI showing the overall topological

similarity. Residues 1 to 230 and 250 to 456 are shown. The NH<sub>2</sub>-terminal domain is in the same orientation as Fig. 1A.

**Fig. 3** Electron density of the final 2.8 Å MIR map contoured at 1.0  $\sigma$  and superimposed on the refined model. The area shown is in the lipid binding pocket of the NH<sub>2</sub>-terminal domain of BPI. The phosphatidylcholine and the surrounding protein atoms are shown.

**Fig. 4A** The covalent structure of phosphatidylcholine and the lipid A region of LPS from *E. coli* and *S. typhimurium*. Phosphate groups are indicated by P. Adapted with changes from [C. R. H. Raetz, *Annu. Rev. Biochem.*, 59:129 (1990)]. **Fig. 4B** Slice through the interior of BPI showing the lipid binding pocket in the NH<sub>2</sub>-terminal domain. The solvent accessible surface of the protein was calculated without lipid present, the interior of the protein and the phosphatidylcholine are shown. Protein residues are shown as ball-and-stick. Figure produced with MSP [M. L. Connolly, *Science*, 221:709 (1983); M. L. Connolly, *J. Am. Chem. Soc.*, 107:1118 (1985)].

**Figs. 5A and 5B** The amino acid sequences of human BPI (SEQ ID NO: 3), LBP (SEQ ID NO: 4), PLTP (SEQ ID NO: 5), and CETP (SEQ ID NO: 6). The alignment was performed with CLUSTAL [D. G. Higgins and P. M. Sharp, *Gene*, 73:237 (1989)] using all eleven known protein sequences from mammals [R. R. Schuman, et al., *Science*, 249:1429 (1990); D. Drayna et al., *Nature*, 327:632 (1987); R. Day et al., *J. Biol. Chem.*, 269:9388 (1994); S. R. Leong and T. Camerato, *Nucleic Acids Res.*, 18:3052 (1990); M. Nagashima, J. W. McLean, R. M. Lawn, *J. Lipid Res.*, 29:1643 (1988); M. E. Pape, E. F. Rehber, K. R. Marotti, G. W. Melchior, *Arteriosclerosis* 11:1759 (1991); G. Su et al., *J. Immunol.*, 153:743 (1994); P. W. Gray et al., *J. Biol. Chem.* 264: 9505 (1989); Albers et al., *Biochem. Biophys. Acta*, 1258:27 (1995); X. C. Jiang et al., *Biochemistry*, 34:7258 (1995); L. B. Agellon et al., *Biochemistry*, 29:1372 (1990); X. C. Jiang et al., *J. Biol. Chem.*, 266:4631 (1991)] but only the four human sequences are shown. Residues that are completely conserved in all proteins are indicated below the sequence \*; those which are highly conserved are indicated by •. The secondary structure of BPI is indicated above the sequences. The  $\beta$  strands are indicated by arrows; strands which make up the central  $\beta$  sheet are shown with gray arrows. Because of the  $\beta$  bulges and pronounced twisting, some of the  $\beta$  strands have one or more residues that do not show classical H-bonding patterns or  $\Phi\Psi$  angles; these

breaks are indicated by ^ above the strands. The  $\alpha$  helices are shown as cylinders, and one-residue breaks in helices B and B' are indicated with a vertical dashed line. The horizontal dashed line indicates the linker region. Peptides from BPI and LBP with the highest lipopolysaccharide-binding activity (Little, et al., J. Biol. Chem. 268: 1865 (1994); Taylor et al., J. Biol. Chem. 270: 17934 (1995)) are in bold italics. The disulfide bond is indicated by S-S. Residues with atoms within 4 Å of the NH<sub>2</sub>-terminal lipid are highlighted with gray shading; residues within 4 Å of the COOH-terminal lipid are shown with white letters in black boxes.

**Please replace the paragraph at page 43, line 27 to page 44, line 6 with the following paragraph:**

To allow insertion of BPI into an optimized mammalian expression vector, a unique *Xho*I site was first added to the 3' end of the BPI gene in pIC108. Two oligonucleotides were synthesized for this purpose: BPI-53 (5' ACT GGT TCC ATG GAG GTC AGC GCC 3') (SEQ ID NO: 7) encoding amino acids 361 - 370 of BPI and BPI-54 (5' GAC AGA TCT CTC GAG TCA TTT ATA GAC AA 3') (SEQ ID NO: 8) encoding the last four amino acids of coding sequence, the stop codon (TGA), and incorporating an *Xho*I site immediately downstream of the stop codon. These oligonucleotides were used to PCR amplify a 280 bp fragment of the C-terminus of BPI and incorporate the *Xho*I site at the 3' end of the gene. The amplified fragment was digested with *Nco*I and *Bgl*II and ligated to a ~4100 bp *Nco*I-*Bam*HI fragment from pIC108 to generate the plasmid pSS101.